RAPID AND QUANTITATIVE SCREENING OF NITROSAMINES IN DRUG PRODUCTS USING HEADSPACE-SIFT-MS

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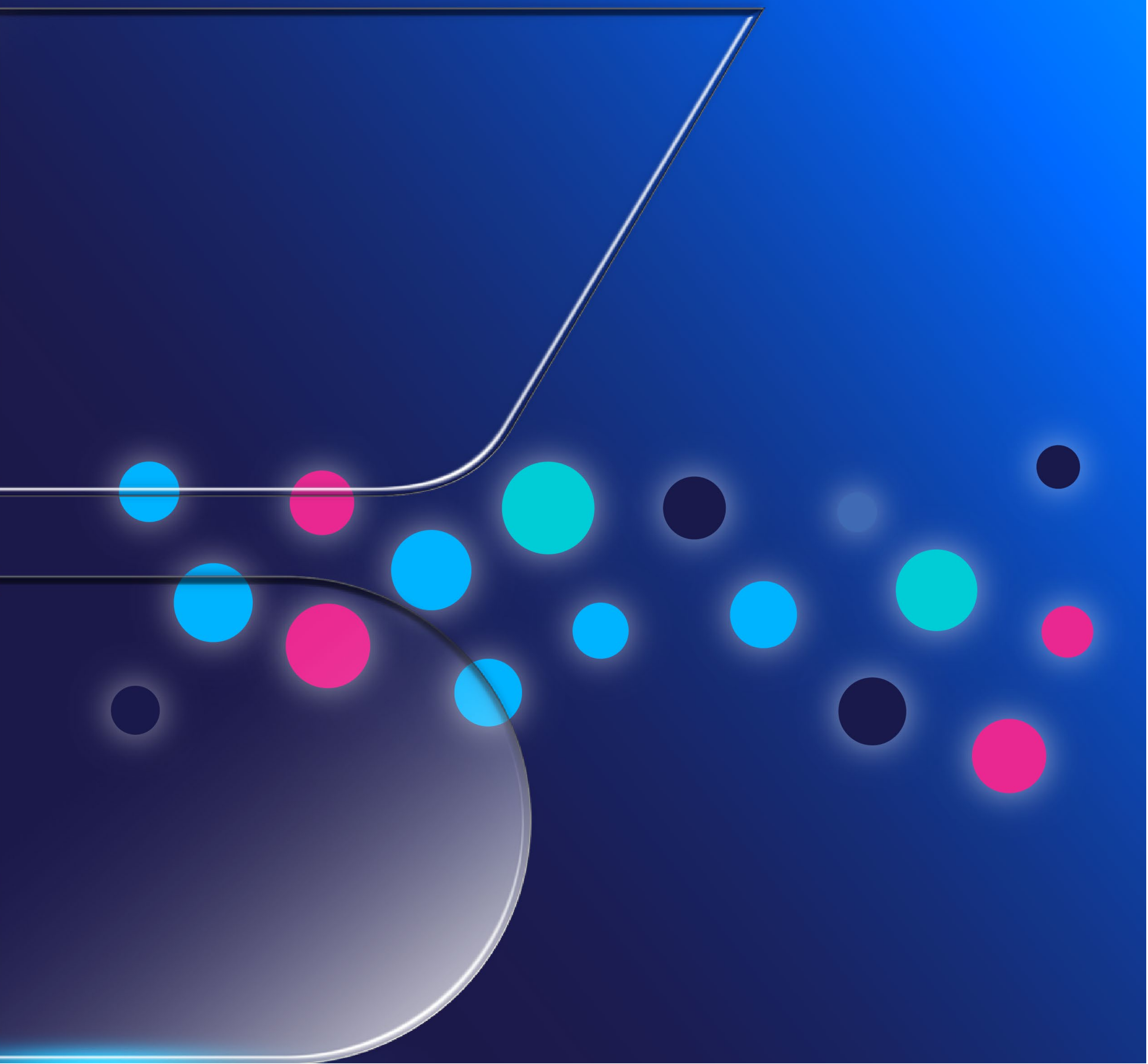

ABSTRACT

Selected ion flow tube mass spectrometry (SIFT-MS) can be used for rapid, selective and highly sensitive detection of nitrosamines in pharmaceutical drug products. This study advances the work done earlier this year (Aitcheson *et al.* (2024)) towards the goal of a fully validated headspace-SIFT-MS method to detect nitrosamine impurities. The acceptance criteria laid out by the United States Pharmacopeia (USP) for method repeatability, intermediate precision, specificity, accuracy, and detectability were met by headspace-SIFT-MS for six nitrosamines. Despite the complex matrix of an over-the-counter drug product, both NDMA and NDEA could be measured at low concentrations with an average recovery rate of 115% and 86% respectively and a limit of quantitation (LOQ) of 0.06 ppm and 0.03 ppm respectively. Compared to conventional chromatographic techniques, SIFT-MS reduces solvent and consumables usage, provides significantly increased sample throughput, and affords unparalleled operational simplicity. The Syft Tracer Pharm11 solution can be used as a screening tool for the detection of nitrosamines and other volatile impurities in pharmaceutical products.

INTRODUCTION

The presence of nitrosamines in pharmaceuticals can pose a significant cancer risk to humans. The US FDA and other global regulatory agencies have issued guidance documents for drug manufacturers to assess and control the presence of nitrosamine impurities in medicines. The acceptable daily intake (AI) limits of nitrosamines, levels that pose a negligible health risk, have been established in pharmaceuticals (US FDA (2019); EMA (2019)). Since exposure to nitrosamines is related to the maximum daily dose (MDD) of a drug, the limit concentration of nitrosamines varies for each material evaluated. The USP general chapter ⟨1469⟩ provides recommended performance criteria for quantitative and qualitative procedures used for the detection of nitrosamines. A user is expected to verify the suitability of quantitative analytical procedures for the specific samples under consideration.

In a recent study, headspace selected ion flow tube mass spectrometry (SIFT-MS), a column-free and organic solvent-free analysis, was reported to provide rapid, sensitive, and selective quantitation of nitrosamines in pharmaceutical drug products. (Aitcheson *et al.* (2024)). The analytical procedure utilizes a simple and fast sample preparation and achieves excellent specificity, linearity ($R^2 > 0.992$) and LOQ (<10% of the individual AI limits) for the six nitrosamines tested. To advance it further, a partial-validation of the headspace-SIFT-MS technique was carried out to assess its ability to meet the recommended quantitative analytical performance criteria described in USP ⟨1469⟩. Repeatability, intermediate precision, specificity, accuracy, and detectability of the headspace-SIFT-MS method to detect nitrosamine were tested. The results discussed in this app note demonstrate that headspace-SIFT-MS fulfils the performance criteria set out by USP and can be used as a screening tool to detect trace nitrosamines in drug products. By meeting the USP criteria this method presents a viable 'green' alternative to the conventional liquid chromatographic (LC-MS) techniques. Headspace-SIFT-MS is faster to run, easier to implement, more cost-effective, and better for the environment than any other nitrosamine analyses available.

METHOD

1. SIFT-MS detection

This work utilized the Syft Tracer Pharm11 solution including a Syft Tracer i3 (Syft Technologies, New Zealand) operating on helium carrier gas, integrated with a multipurpose autosampler (PAL RSI, CTC Analytics AG, Switzerland) and operated using the SyftAuditTracer software (Syft Technologies, New Zealand). The fundamentals of SIFT-MS and details of the SIFT-MS methods used to monitor the test nitrosamines are described in Langford (2023a) and Aitcheson *et al.* (2024), respectively.

2. Sample preparation

Master stock solution: Neat certified reference materials (CRM) of *N*-Nitrosodimethylamine (NDMA) and *N*-Nitrosodiethylamine (NDEA) were acquired from Supelco™ (purity 97.3% and 99.8% respectively). Two separate master stock solutions (~1000 µg/mL) were prepared by adding 5 µL of the neat CRM to a 5 mL volumetric flask and making it to volume with deionized water. For the remaining test analytes, approx. 1 mg/mL nitrosamine in methanol CRM solutions were acquired and used as master stock solutions (Table 1):

Test analyte	***Concentration (µg/mL)***
N-Nitrosoethylisopropylamine (NEIPA)	1050.3
N-Nitrosodiisopropylamine (NDIPA)	999.8
N-Nitrosomethylphenylamine (NMPA)	947.4
N-Nitrosodibutylamine (NDBA)	990.8

Table 1. Certified reference standards of nitrosamines in methanol.

Working stock solution: For each test analyte, working stock solutions (~0.1 µg/mL) were prepared by adding 5 µL of the respective master stock solution to a 5 mL volumetric flask and making it to volume with deionized water.

Test solution: All calibrants, blanks and samples were prepared in 10 mL amber headspace vials. All vials used were first prepared by adding 5 ± 0.1 g of EMSURE® potassium carbonate (K_2CO_3), purity ≥ 99.0%. Test solutions were prepared as follows:

1. Repeatability:
    a. NDMA: The method repeatability for NDMA was determined using vials containing a mass of 9.6 ng (10% of the AI limit) in each test vial. The repeatability test solution was prepared by adding 219.3 µL of the working stock to a 100 mL volumetric flask and made to volume with deionized water. Twelve replicate vials were prepared by aliquoting 4.5 mL of the freshly prepared repeatability solution to vials pre-filled with K_2CO_3.
    b. Method repeatability for the rest of the nitrosamines was determined by preparing 6 replicate vials, each containing 5 ng (5 µL of respective working stock) of the individual nitrosamine in 5 mL of aqueous K_2CO_3 solution, saturated at room temperature.
2. Intermediate precision: The method's intermediate precision was determined by repeating the above procedure for repeatability on two further occasions. A fresh test solution was prepared each day.
3. Recovery: Calibration standards of NDMA and NDEA were prepared separately (Table 2).

Calibrant	***Working stock volume (µl)***	***Mass K_2CO_3 (g)***	***Deionized water volume (mL)***	***Mass NDMA (ng) in test vial***	***Mass NDEA (ng) in test vial***
Cal 0	0	5 ± 0.1	4.5	0	0
Cal 1	1	5 ± 0.1	4.5	0.98	0.94
Cal 2	2.5	5 ± 0.1	4.5	2.44	2.35
Cal 3	4	5 ± 0.1	4.5	3.91	3.76
Cal 4	7.5	5 ± 0.1	4.5	7.33	7.05
Cal 5	10	5 ± 0.1	4.5	9.78	9.40

Table 2. Calibration standards for NDMA & NDEA recovery.

The Article Under Test (AUT) in this study was a finished drug product containing fexofenadine hydrochloride (MDD – 180 mg), a common antihistamine sold under the brand name Telfast®. Blank and nitrosamine (NDMA and NDEA) spiked solutions were prepared according to Table 3. Test solutions were prepared by aliquoting appropriate volumes of blank or spiked solutions to headspace vials pre-filled with K_2CO_3 (Table 3).

Components	***Blank solution NDMA***	***NDMA spiked solution***	***Blank solution NDEA***	***NDEA spiked solution***
AUT (mg)	180	180	360	360
Working stock (μL)	–	98.2	–	28.1
Mass of nitrosamine in flask (ng)	–	96	–	26.5
Concentration (nitrosamine/AUT) (ppm)	–	0.533	–	0.072
Total volume (mL)	100	100	100	100
Nitrosamine solution conc. (ng/mL)	0	0.96	0	0.265
Vial aliquot volume (mL)	4.5	4.5	4.5	4.5
Mass nitrosamine/vial (ng)	0	4.3	0	1.2
Replicates	3	9	3	9

Table 3. Sample preparation for NDMA & NDEA recovery.

3. Automated sample delivery

Samples were incubated at 80 °C for 15 minutes in an agitator. Headspace (2.5 mL) was extracted using a syringe (heated to 120 °C) and subsequently injected into the analyzer at a flow rate of 120 μL/s. Since the nominal sample flow into the SIFT-MS instrument is 420 μL/s, a make-up gas flow (clean dry air) is also introduced through the sampling head. After each injection, the syringe needle was flushed with clean dry air for 60 seconds. The advanced scheduling feature of the autosampler enabled analysis of 15 samples in 90 minutes.

RESULTS AND DISCUSSIONS

The sample preparation using the dissolution approach eliminates the usage of organic solvents improving the sustainability and economic returns of the analytical process. LC-MS/MS – commonly utilized to detect NDMA, utilizes a gradient elution (starting with 97% water and 3% methanol, both elution solvents contained 0.1% formic acid, followed by a ramp to 95% methanol for 9 minutes and held for 3 minutes) (Procedure 3, United States Pharmacopeia). Water, an ideal solvent for SIFT-MS, is a safe, non-toxic, non-flammable, cost-effective, and sustainable alternative to traditional hazardous organic solvents such as methanol used in LC-MS/MS detection of nitrosamines.

Conventional chromatographic techniques consume high set-up or conditioning times, are slow to report and have low daily throughputs. Syft Tracer Pharm11 solution addresses these challenges through a single stable instrument configuration that requires very little set-up between applications, infrequent calibration and is easy to operate. This means that the time to the first analytical result is significantly reduced and daily sample throughput is enhanced multi-fold compared to conventional techniques. Langford *et al.* (2023c) describe how SIFT-MS provides a very flexible analytical platform for the analysis of five dissimilar applications using a single instrument configuration and replace multiple chromatographic systems.

The precision of the analytical procedure or repeatability is reported as a percentage relative standard deviation (% RSD) of measurements of six replicate samples prepared from the same homogenous stock solutions. Repeatability using SIFT-MS for all six nitrosamines included in this study was excellent (<8% RSD; Table 4) and meets the recommended acceptance performance criteria (% RSD ≤ 25%) as per USP ⟨1469⟩.

Hence measurements made using Syft Tracer Pharm11, under identical conditions, produce repeatable results with minimal variability across different test analytes.

Test analyte	Repeatability (% RSD)	Intermediate Precision (% RSD)
NDMA	4%	13%
NDEA	2%	22%
NEIPA	8%	13%
NDIPA	2%	3%
NMPA	5%	31%
NDBA	5%	39%

Table 4. SIFT-MS performance data for repeatability (% RSD, n= 12 (NDMA) or 6 (remaining five nitrosamines)) and intermediate precision (% RSD, n=18) for six test nitrosamines.

Intermediate precision is reported as % RSD calculated from the standard deviation of the mean value from each run divided by the grand mean (n=18). The intermediate precision listed was calculated using the instrument response without external calibration, the values therefore encompass differences in measurements arising from changes in environmental factors, instrumental conditions and uncertainties associated with sample preparation. The acceptance criteria (%RSD ≤ 30%) were comfortably met for four out of six test nitrosamines (Table 4) showing that test samples prepared and analyzed using SIFT-MS on different days show good precision and little variability across batches. The precision of NMPA and NDBA suffered due to poorer headspace partitioning and lower instrument sensitivity as the intensity of the response was up to ten times less than the other species tested. Improvements in sensitivity can be made by using a higher injection speed and a greater volume headspace syringe.

Spike and recovery study is used to determine whether analyte detection is affected by a difference between the diluent used to prepare the standard curve and the drug product matrix. The recovery of two nitrosamine species, NDMA and NDEA, is demonstrated here. The recovery study for NDMA, the most commonly reported nitrosamine impurity, was performed at the limit concentration, defined as:

$$Limit\ concentration\ (ppm) = \frac{Acceptable\ Intake\ (ng/day)}{Maximium\ Daily\ Dose\ (mg)}$$

The recovery of NDEA, which is more potently carcinogenic, was performed at half of the limit concentration. The recommended AI of NDMA and NDEA is 96 ng/day and 26.5 ng/day respectively. Fexofenadine was chosen as the AUT due to its high water solubility, easy availability over the counter, and because at 180 mg it has a midrange MDD. The chosen volume of water was 100 mL to ensure that the entire mass was solvated. Should this volume be minimized, the accuracy and precision would improve as the effective amount of analyte being measured moves further from the LOQ.

The correlation between the nitrosamine relative headspace response (ppb) and the standard concentration was linear from 1 to 10 ng. The intercept, slope and correlation coefficients of the linear curve were determined. The nitrosamine concentration in the spiked drug product was calculated using the formula below:

$$R_2N_2O\ (ppm) = [(y-c)/m]\ \div wt.$$

Where:
y = relative headspace response
c = intercept of the linear curve
m = slope of the linear curve
wt. = weight of the drug product in the sample vial

$$Recovery\ \% = \frac{measured\ concentration}{expected\ concentration} \times 100$$

Please note that the test matrix is not an isolated active pharmaceutical ingredient, but a finished drug product with several other ingredients which could potentially interfere with the analysis. In addition to fexofenadine hydrochloride, the inactive ingredients in Telfast® are as follows:

> *microcrystalline cellulose, pregelatinised maize starch, croscarmellose sodium, magnesium stearate, povidone, titanium dioxide, colloidal silicon dioxide, macrogol 400, hypromellose, Pigment Blend Pink PB1254, Pigment Blend Yellow PB1255 (Medsafe, NZ).*

Despite the complexity of the test matrix the SIFT-MS method managed to selectively detect NDMA and NDEA at very low levels. Selectivity is aided by the utilization of multiple product ions, which arise from distinct reaction mechanisms, for quantitation. In cases where interference occurs at all product ions available, such as the ^{13}C isotopologue of *N,N*-dimethylformamide which is known to interfere with NDMA, a simple subtraction can be performed (Langford *et al.* (2023b)). With the exception of a single outlier, the analysis met the USP ⟨1469⟩ requirements i.e., recoveries between 70 – 130% (Table 5). The single outlier is only slightly higher (132%) than the desired target recovery percentage. With a multi-component matrix such as this, other techniques variously require filtration, preconcentration, derivatization or chromatographic separation (Guiraldelli (2019)), whereas SIFT-MS can forgo all these and achieve the requisite sensitivity and selectivity directly from aqueous headspace.

Replicate	***NDMA Concentration (ppm)***	***NDMA Recovery***	***NDEA Concentration (ppm)***	***NDEA Recovery***
1	0.616	115%	0.063	85%
2	0.622	117%	0.059	80%
3	0.643	121%	0.056	76%
4	0.509	95%	0.067	91%
5	0.596	112%	0.059	80%
6	0.573	107%	0.085	115%
7	0.705	132%	0.055	75%
8	0.559	105%	0.056	76%
9	0.621	116%	0.067	92%
Average	0.614	115%	0.063	86%
% RSD	9%		14%	

Table 5. Recovery data for NDMA and NDEA from spiked solutions of fexofenadine *(brand name:* Telfast® *).*

The signal-to-noise ratio (S/N), the theoretical limit of detection LOD, and LOQ, specific to the sample preparation performed on the fexofenadine sample, for headspace-SIFT-MS analysis of NDMA and NDEA is summarized in Table 6. The LOQs for NDMA and NDEA are estimated to be 0.06 ppm and 0.03 ppm, respectively.

Analyte	***Concentration (ppm)***	***Signal-to-noise ratio (S/N)***	***Theoretical LOD Based on 3:1 S/N (ppm)***	***Theoretical LOQ Based on 10:1 S/N (ppm)***
NDMA	0.533	105	0.02	0.06
NDEA	0.072	32	0.008	0.03

Table 6. The signal-to-noise ratio (S/N), the theoretical limit of detection LOD, and LOQ for headspace-SIFT-MS analysis of NDMA and NDEA in a 2.5 mL aliquot injected at 120 µL/s.

CONCLUSIONS

- SIFT-MS meets the performance acceptance criteria laid out by USP ⟨1469⟩ for the detection of nitrosamines.
- Headspace-SIFT-MS provides a rapid, highly selective, linear and sensitive analysis of six commonly reported nitrosamines.
- The SIFT-MS method can detect NDMA with high specificity in the presence of known interferent compounds (DMF) in complicated matrices.
- Despite the complicated matrix of a finished drug product, SIFT-MS provided highly sensitive detection of NDMA and NDEA at low concentrations.
- A single configuration of the Tracer Pharm11 solution provides analytical flexibility to seamlessly switch between applications. This capability reduces instrument downtime, redefines analytical productivity and enhances throughput.

ACKNOWLEDGEMENT

We are grateful to Dr Mark Perkins, Element Materials Technology Laboratory Instrumentation, Cambridge, United Kingdom and Dr Vaughan Langford, Syft Technologies Limited, Christchurch, New Zealand for their technical advice during method development.

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